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BIOMEDICAL RESEARCH INST ROCKVILLE MD

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PRODUCTION OF MALARIA VACCINE BY ARTIFICIAL FEEDING OF MOSQUITO--ETC(U)

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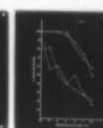
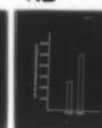
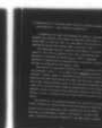
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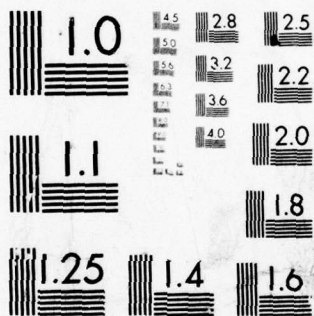


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Office of Naval Research

Contract No. ¹⁵ ~~N00014-76-C-1132~~ ^{new}

Task No. NR-204-053

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⁶ Production of Malaria
Vaccine by Artificial Feeding
of Mosquitoes.

by

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-Abstract-

The report is divided into three chapters.

(1) Infection of mosquitoes by feeding under artificial conditions. Experimental requirements for artificially feeding mosquitoes are described along with the necessary special apparatus which was designed and built in our laboratory. (2) Determination of the best mouse strain for carrying gametocytes and production of high numbers of sporozoites. It is demonstrated that the NMRI mouse is clearly the best choice for proliferation of both these stages of the Plasmodium berghei parasites. (3) Determination of Exoerythrocytic stages of P. berghei. Preliminary experiments involving tissue culture of embryonic rat tissue where sporozoites develop into mature schizonts are described.

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NYB	White Section
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Our work with gametocytes has involved the use of other stages in the life cycle of Plasmodium berghei. Although the results obtained will have a direct bearing on the gametocyte program, the initial feasibility was more easily determined on stages other than the gametocyte.

This report is divided into the following chapters:

- 1- Infection of mosquitoes by feeding under artificial conditions.
- 2- Determination of the best mouse strain for carrying gametocytes and production of high numbers of sporozoites.
- 3- Demonstration of exoerythrocytic stages of P. berghei.

1) Artificial Feeding

We have designed and built a chamber which maintains donor blood and serves as both a site and a source for mosquitoes to obtain a blood meal artificially (Figure 1). This apparatus has proven to be essentially trouble free during operation. The chamber is made of plastic and has the overall dimensions of 6x4 cm. It is a dual size feeding chamber having one feeding site, (blood well) 3 cm in diameter (blood volume 0.5 ml) while the other is 4.5 cm in diameter (blood volume 1.0 ml). The chambers are fitted with artificial membranes (condom material) or the Baudruch membrane held in place with a "o" ring. The chamber is maintained at a constant temperature of 37°C by means of circulating water from a Master-line circulating water bath (Forma, Scientific, Model 2095). The blood to be used for artificial feeding is introduced via the blood filling port (Fig. 1) with a syringe equipped with a 20g needle. Blood meals were given in the insectary at 21°C. Multiple chambers may be connected in a series so that several cages of mosquitoes can be fed simultaneously. The blood for these experiments was used when the donor mice had between 2-4% gamétocytes as determined from Giemsa's stained blood films. Before each artificial feeding, control mosquitoes were fed a blood meal from donor mice just prior to withdrawing blood (exsanguination via heart puncture) for artificial feeding. The blood was defibrinated prior to its addition to the feeding chamber by one of several different methods such as outlined by Yoeli et al. (1963) and Carter and Nijhout (1977). We have also

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examined the effect of defibrinating whole blood alone or in several different buffered solutions. The blood in the latter cases was added dropwise to approximately 5 ml of defibrinating solution contained in a 30 ml beaker while being gently stirred with a magnetic stirrer. Stirring was continued for 2-3 minutes and then the contents were transferred to a 10 ml conical centrifuge tube, with a Pasteur pipette and centrifuged at 1000 x g for 10 minutes, resuspended in the appropriate volume of supernatant and then loaded into the feeding chamber as outlined above. A small portion of this blood was retained for exflagellation studies. A drop of this blood was placed on a slide, covered with a coverslip and sealed with petroleum jelly. The preparation was then observed at 21°C for exflagellation under a 40x objective using phase contrast microscopy.

Mosquitoes: *Anopheles stephensi*

Mosquitoes were reared in an insectary maintained at 27°C with a relative humidity of 75-80%. For feeding and parasite development, the adult mosquitoes were moved to another insectary at 21°C, the same relative humidity as above. Food and water were removed 24 hours prior to a blood meal.

Plasmodium berghei parasites

P. berghei, anka strain malaria parasites are maintained in female NIH/NMRI white mice. Our malaria line is maintained by a return to a mosquito passage after every second blood passage.

Infectivity of Blood

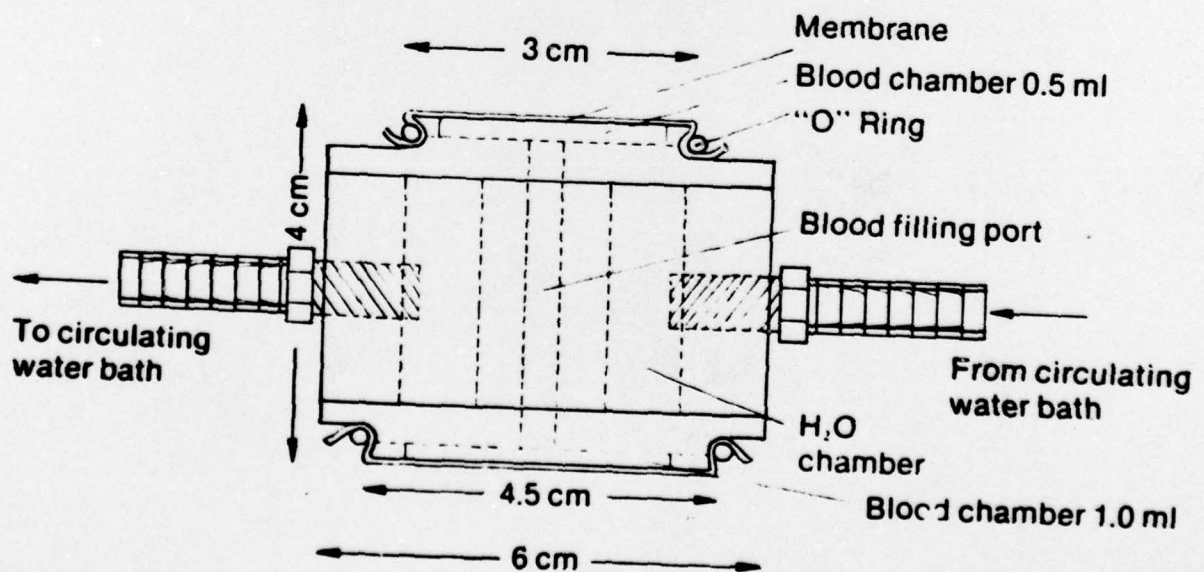
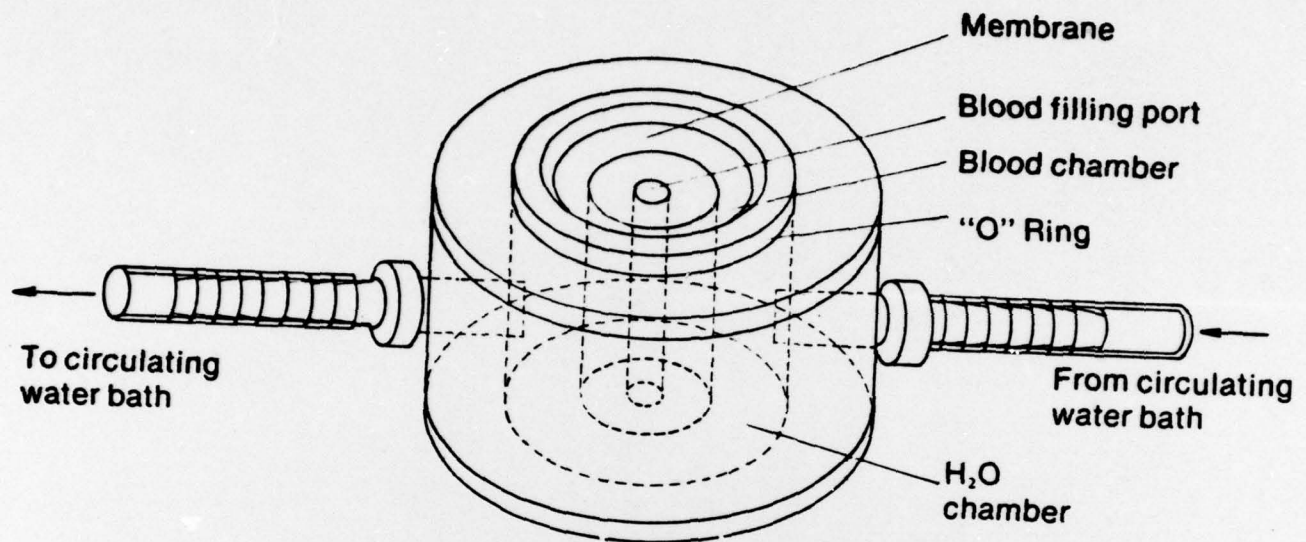
The infectivity of the blood artificially fed to mosquitoes was determined by the presence of oocysts in the stomach of the mosquitoes as determined by dissection and observation under a magnification of 400x, nine days after the blood meal.

Results and Discussion

The controls indicate that the blood is infective before being drawn for artificial feeding experiments, and exflagellation studies show that microgametocytes are active at the time the blood is added to the chamber. Nevertheless, we have recorded only 2% of the mosquitoes as being infected with this blood. The only way the parasites can infect the mosquito is via the gametocytes thus it is clear that these forms are not stable in the solutions thus far tested. Since we have had largely negative results with defibrinated whole blood at various temperatures and in variously buffered solutions, we plan to examine the ability of low temperature preservatives to maintain the integrity of gametocytes. It is known that such compounds stabilize biological material. We will initially use those compounds which have proven successful in our program involving the preservation of sporozoites. The solutions and or additives are: whole mouse serum, polyvinyl pyrrolidone, hydroxyethyl starch, glycerol and Dimethyl sulfoxide.

Figure 1

Dual-Size Chamber for Feeding Mosquitoes



2) Determination of the best mouse strain for carrying gametocytes and production of high numbers of sporozoites.

A comparison was made first between the number of sporozoites produced per mosquito after having an infective blood meal on either NMRI or Flow mice (Fig. 1); and second, the infectivity of sporozoites derived from either NMRI or Flow mice (Fig. 2).

The mosquitoes were fed infective blood meals on one mouse strain or the other as described above while sporozoites were harvested as described by Pacheco et al. (1978). Subsequent handling of sporozoites and determination of sporozoite infectivity was done as described by Leef et al. (1978).

The results of sporozoite production on a per mosquito basis are presented in Fig. 1. It is clear the A. stephensi mosquitoes produced a greater number of sporozoites when the infective blood meal was from NMRI mouse donors than when from Flow mice. Mosquitoes fed on NMRI donors produced nearly 26,000 sporozoites/mosquito while those fed on the Flow donors produced approximately 13,000 sporozoites/mosquito. Since the system is two times more efficient using NMRI donors, we plan to continue work with that strain of mouse in the future.

The results of the comparison between the infectivity of sporozoites injected into either NMRI or Flow mice where the mosquitoes giving rise to the sporozoites were infected from either NMRI or Flow mice, are presented in Table 1 and Figure 2. At every dose level, sporozoites are more infective in the NMRI mouse. A compar-

ison of the 50% effective dose (ED_{50}) for NMRI versus Flow host mouse is 500 compared to 6,000 respectively. Thus, it is clear that the NMRI mouse is the host of choice in such a study.

Figure 1

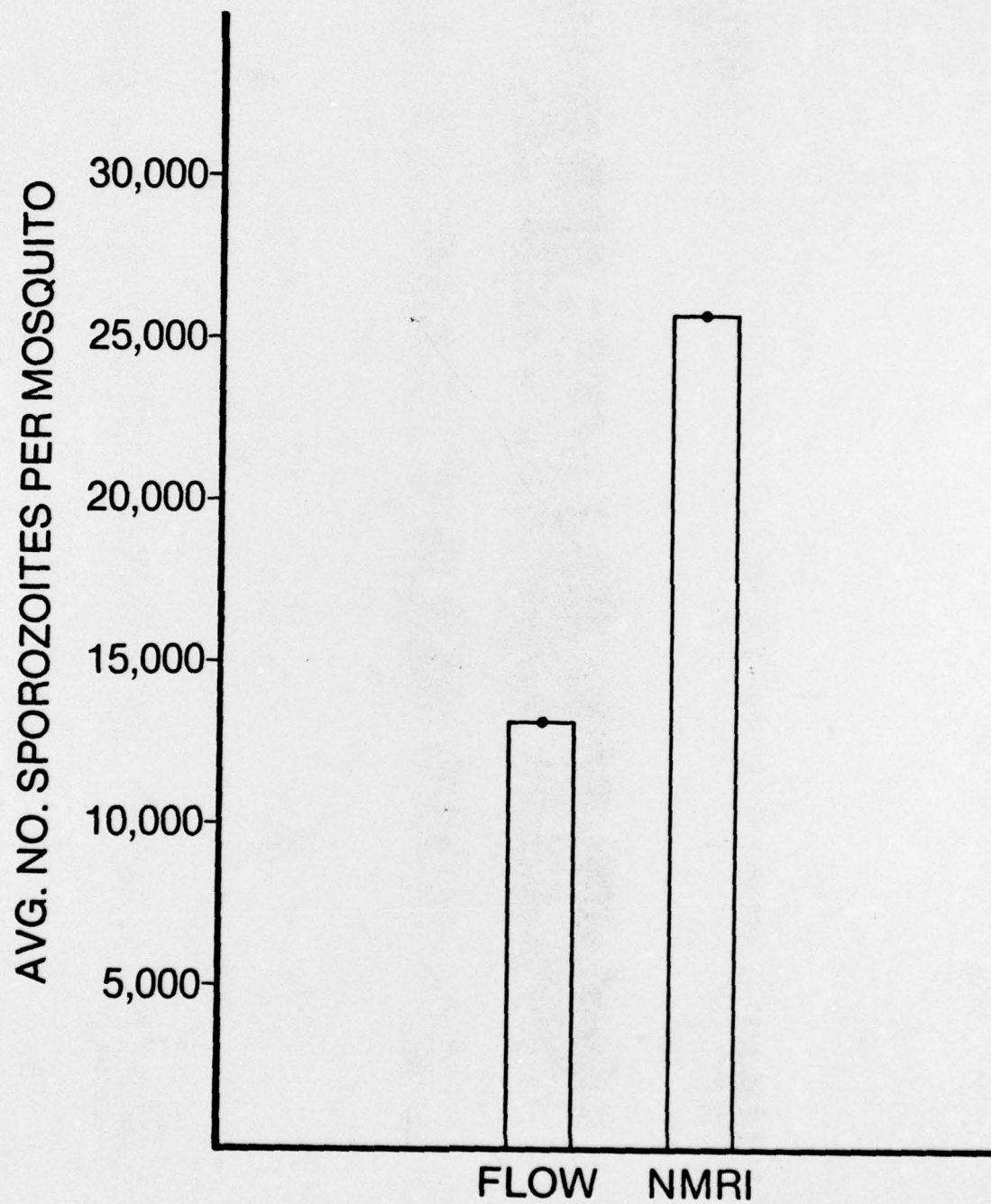


Figure 2

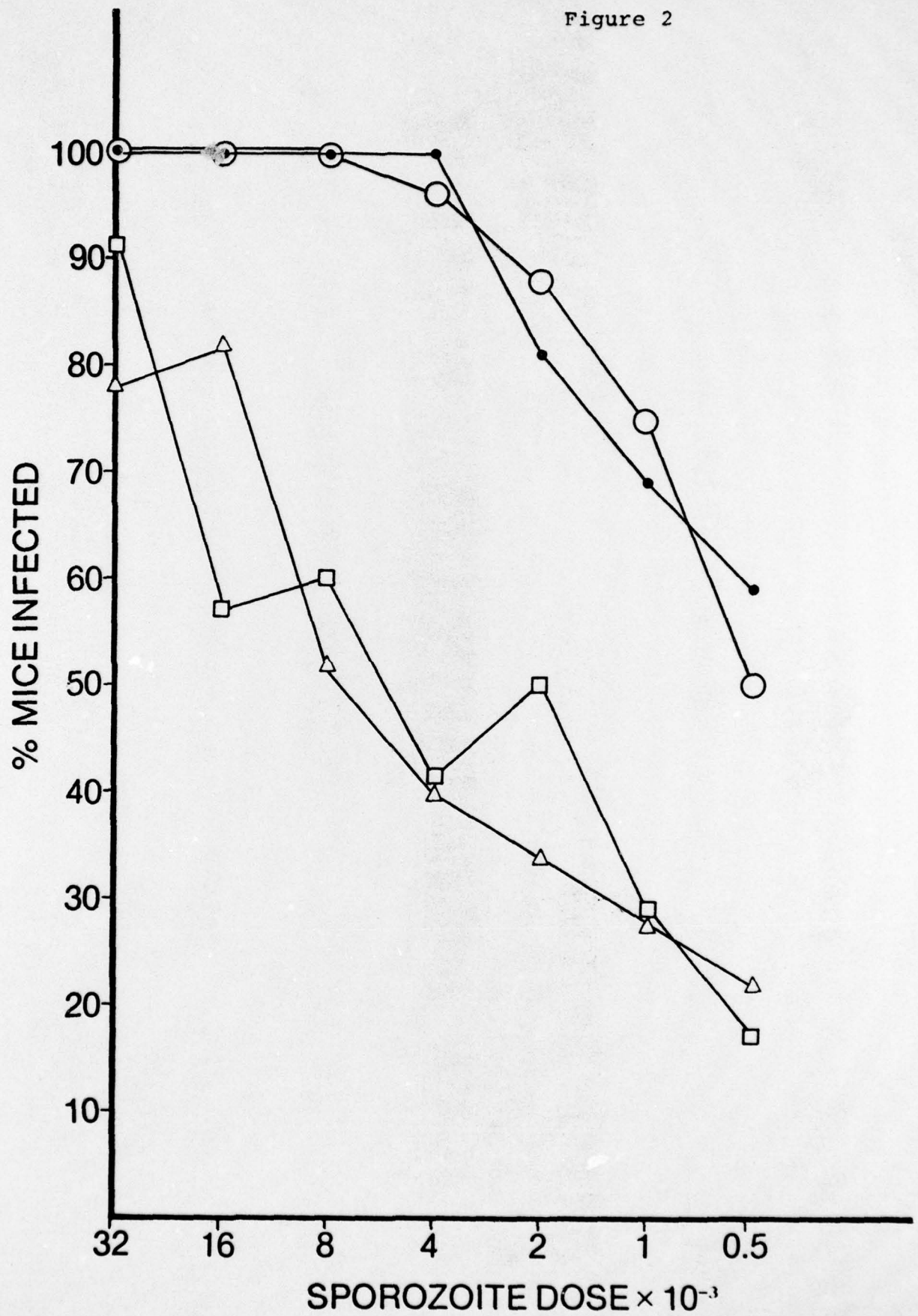


Table 1

RECIPIENT MOUSE

Source of Infection	NMRI % Infected										Flow Infected									
	Dose IV																			
	32,000	16,000	8,000	4,000	2,000	1,000	500	32,000	16,000	8,000	4,000	2,000	1,000	500						
Mosquitos-Flow Infected	100	100	100	100	100	70		100	78	43	50	38	40							
	100	100	100	100	88	86	50	56	100	63	57	25	43	44						
	100	100	100	100	56	50	66	78	50	50	14	38	0	0						
	100±0 ^a	100±0	100±0	100±0	81±22	69±18	58±11	78±22	82±24	52±10	40±23	34±8	28±24	22±31						
Mosquitos-NMRI Infected	100	100	100	100	100	100	20	88	25	75	43	29	17	10						
	100	100	100	100	100	100	90	100	71	75	50	71	57	40						
	100	100	100	88	63	25	40	86	75	29	29	50	14	0						
	100±0	100±0	100±0	96±7	88±21	75±43	50±36	91±8	57±28	60±27	41±11	50±21	29±24	17±21						

^a Mean and standard deviation.

-References-

1. Pacheco, N.D., C.P.A. Strome, F. Mitchell, M.P. Bawden and R.L. Beaudoin. Rapid, large-scale isolation of Plasmodium berghei sporozoites from infected mosquitoes. J. Parasit. (In Press). (1979).
2. Leef, J.L., C.P.A. Strome and R.L. Beaudoin. The low temperature preservation of sporozoites of Plasmodium berghei. Bull. W.H.O. (In Press). (1978).

3) Demonstration of exoerythrocytic stages of P. berghei malaria.

In a preliminary series of experiments, we have infected a monolayer of embryonic rat brain tissue with sporozoites. The developmental cycle then proceeded from the sporozoite to the mature schizont but the cycle has not gone beyond that point nor has it lasted longer than one generation. Although we have had many cultures proceed to the schizont stage, the technique is difficult to reproduce. Thus far, we have been unable to infect mice with these tissue stages.

Animal Care and Utilization Report
Production of Malaria Vaccine by Artificial Feeding of Mosquitoes
for
ONR Contract N00014-76-C-1132

The malaria studies in this report were performed on mice, rats and guinea pigs using an established laboratory adopted strain of rodent malaria P. berghei. The animals were cared for and utilized in compliance with the Animal Services Procurement Regulations.

The treatment of the animals and the drugs are listed below:

1. 312 mice were involved in procedures that caused no pain.
2. 150 mice were involved in procedures producing pain, and were given anesthetic to immobilize and to relieve the discomfort of feeding mosquitoes (Sodium Pentobarbital 0.2 mg/gram of wt. IP)
3. No animals were involved in procedures that produced pain where an anesthetic was not given.

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